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(54) Title: LACCASE AND BEER STORAGE (57) Abstract The present invention relates to a beer-making process comprising fermenting wort into beer and adding a laccase to the fermented beer so as to improve the storage stability of the beer.		

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LACCASE AND BEER STORAGE

FIELD OF INVENTION

The present invention relates to the use of microbial laccase in order to produce a storage stable beer.

5 BACKGROUND OF THE INVENTION

Storage life of a beer depends on many factors, e.g. temperature, haze forming potential and oxygen content.

Classic haze formation in beers is a result of protein precipitation that is usually stimulated by small
10 quantities of naturally occurring proanthocyanidin polyphenols. This type of complex is frequently manifested as chill-haze, which appears on cooling but which may re-dissolve at room temperature or above. This is generally ascribed to hydrogen-bonding or to hydrophobic interactions with proline residues.
15 At a later stage, nucleophilic substitution of phenolic rings by protein sulphydryl groups may lead to a permanent haze that does not re-dissolve when warmed.

The excess polyphenols may traditionally be removed by treatment with insoluble polyvinylpolypyrrolidone (PVPP).
20 PVPP is a dusty powder which is difficult to handle without creating an unhealthy environment for the workers; PVPP is also a problem in the waste water due to its low biodegradability. Alternatives to PVPP are needed.

To reduce the amount of polyphenols it has been
25 suggested to add polyphenol oxidase, e.g. laccase, to the wort, for reference see Food Biotechnology, Vol.3 no.2, 1989, pp. 203-213 and US 4,411,914.

Most of the chemical changes during storage of beer involve oxidation. The chemical changes will therefore be
30 accelerated if the beer is allowed any contact with oxygen after it leaves the fermentation vessels. The development of "Ribes" or catty taint in bottled beer is strongly correlated with the amount of air in the headspace, for reference see J.

Inst. Brew. 82, 1976, p. 175. Roughly 1 ml of air in a 300 ml bottle will give an oxygen content of 1 ppm. This amount is probably sufficient to oxidize all the reductones present in a light lager beer. The dissolved oxygen in beer rapidly disappears, usually without the immediate formation of an off-flavour, but the damage may have been done as beer contains compounds such as melanoidins and reductones which act as oxygen carriers capable of producing off-flavours at a later date. Antioxidants such as sulphur dioxide or ascorbic acid are sometimes added to beer but these antioxidants can cause other problems, for reference see Malting and Brewing Science Vol II, 1991, pp. 872-873.

It is the purpose of the present invention to improve the storage stability of beer by both reducing the oxygen and the polyphenol content.

SUMMARY OF THE INVENTION

In this invention it is surprisingly found that a storage stable beer may be produced by adding laccase to the fermented beer.

Accordingly, the present invention relates to a beer-making process, comprising

- fermenting wort into beer, and
- adding a laccase to the fermented beer so as to improve the storage stability of the beer.

DETAILED DESCRIPTION OF THE INVENTION

Laccase

According to the invention microbial laccase (EC 1.10.3.2) is preferred because it may be dosed very precisely. The microbial laccase may be derived from bacteria or fungi (including filamentous fungi and yeasts). The microbial laccase is preferably obtained from a fungus.

Some preferred fungi include strains belonging to the subdivision Basidiomycotina and to the subdivision Ascomyco-

tina. Suitable examples include a laccase derivable from a strain of Aspergillus, Neurospora, e.g., N. crassa, Podospora, Botrytis, Collybia, Fomes, Lentinus, Pleurotus, Trametes, e.g., T. villosa and T. versicolor, Rhizoctonia, e.g., R. solani,
5 Coprinus, e.g., C. plicatilis and C. cinereus, Psatyrella, Myceliophthora, e.g., M. thermophila, Schytalidium, e.g., S. thermophilum, Polyporus, e.g., P. pinsitus, Phlebia, e.g., P. radita (WO 92/01046), Coriolus, e.g., C. hirsutus (JP 2-238885), Hygrophoropsis, Agaricus, Vascellum, Crucibulum,
10 Myrothecium, or Sporormiella.

In particular laccases derivable from T. villosa, T. versicolor or M. thermophila are preferred.

The laccase may furthermore be one which is producible by a method comprising cultivating a host cell
15 transformed with a recombinant DNA vector which carries a DNA sequence encoding said laccase as well as DNA sequences encoding functions permitting the expression of the DNA sequence encoding the laccase in a culture medium under conditions permitting the expression of the laccase and
20 recovering the laccase from the culture.

Determination of Laccase Activity (LACU)

Laccase activity is determined from the oxidation of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) by oxygen. The greenish-blue colour produced is photometered at
25 418 nm. The analytical conditions are 1.67 mM ABTS, 0.1 M phosphate buffer, pH 7.0, 30°C, 3 minutes reaction.

1 laccase unit (LACU) is the amount of enzyme that catalyses the conversion of 1 μ mole ABTS per minute at these conditions.

30 Production of Beer

Making beer is a complicated process. The process may be outlined in the following way:

The starting material is barley which is malted (i.e. dampened, germinated and subsequently dried). The malt is
35 grounded and mixed with water, heated and stirred. This mixture

is then filtered. After addition of hops, the so called beer wort is boiled. Hereby a precipitation of some of the polyphenols will take place. After removing the precipitates by filtration or other means of separation, the finished beer wort is aerated to typically 8-10 ppm oxygen and yeast is added.

After a main fermentation, lasting typically 5-10 days, most of the yeast is removed and the so called green beer, containing yeast cells, is stored at a low temperature, typically 0°C to 5°C during one to twelve weeks. During this period yeast will precipitate together with polyphenols. To remove the remaining excess polyphenols polyvinylpyrrolidone (PVPP) or another useful material may be added. After one more filtration the beer is ready to be bottled and stored.

15 Application of Laccase

According to the invention laccase is added at the end of the process, because oxygen is unwanted in the finished beer, so addition of laccase may remove any excess oxygen whereby storage life of the beer is enhanced, and at the same time the laccase will remove some of the polyphenols that may still remain in the beer. A suitable amount of laccase is in the range of from 0.1-1000 LACU per liter of fermented beer, preferably in the range of from 1-50 LACU per liter of fermented beer. The polyphenol complexes formed by laccase may be removed by filtration or other means of separation.

In addition to laccase a reduced amount of polyvinylpyrrolidone may also be added to the fermented beer.

Storage Stability Tests

Storage stability tests may be performed in many different ways; a quick way of predicting shelflife may be performed as follows (for reference see Analytica-EBC 1987, 9.17, Prediction of Shelflife): For determining initial haze the turbidity of the beer is measured at 0°C. The sample is then stored at 60°C for 48 hours whereafter the sample is cooled and kept at 0°C overnight. For determining final haze the

turbidity is measured at 0°C. The results are reported in EBC formazin units.

By using the process of the invention the amount of "permanent haze" may be reduced.

5 The following example further illustrates the present invention, and it is not intended to be in any way limiting to the scope of the invention as claimed.

EXAMPLE 1

Reduction of Oxygen Content in Finished Beer

10 Materials and Method

Beer: Pilsner type beer stabilized normally using PVPP, 10.7%P, 4.6% v/v alcohol.

Enzyme: Laccase SP 710 (available from Novo Nordisk A/S, product former called Laccase SP 504; a Trametes villosa laccase)
15

Bottle turner: 16 turns pr. minute.

Sampling device: LG Automatic

Oxygen Meter: DIGOX EC-401.

Beer Treatment:

20 Laccase was diluted with oxygen-free water to a concentration of 25 x 2/3 and 50 x 2/3 LACU/ml, respectively. The beers were cooled to 0°C, opened, and ½ ml of the enzyme solutions or oxygen-free water was added with a syringe resulting in beers with 0, 25 and 50 LACU/liter beer, respectively. After the enzyme addition the beer bottles were knocked
25 at until a foam plug had developed, then air was added using a syringe, in the amounts of 0, 3 and 6 ml, respectively. Hereafter the bottles were crowned, turned for 10 minutes and

stored for 1 hour or 24 hours at 0°C.

Oxygen Analysis:

The beer bottles were turned by hand 10 times before placed in the sampling device where the crown was penetrated and the beer pressed through the oxygen meter by CO₂ at a flow rate of approx. 9 liter/hour. The oxygen content in mg/liter was measured using the DIGOX EC-401. For all treatments triple determinations were carried out.

Results:

10 The oxygen content in 5 untreated beer gave the result of 0.07 +/- 0.03 mg oxygen/liter.

In order to detect if adding oxygen-free water to the beer had any effect on the oxygen content, ½ ml oxygen-free water was added to the beer; a triplicate result showed an
15 oxygen content of 0.05 +/- 0.01 mg oxygen/liter, demonstrating that there is no increase in the oxygen content.

Table 1

Oxygen determinations. (mg oxygen/liter)

	Beer + water		Beer + 25 LACU/ liter		Beer + 50 LACU/liter	
	1 hour	24 hours	1 hour	24 hours	1 hour	24 hours
3 ml air/ bottle	1.06 ± 0.15	1.22 ± 0.23	1.04 ± 0.10	0.08 ± 0.03	0.15 ± 0.03	0.11 ± 0.06
6 ml air/- bottle	2.38 ± 0.12	2.33 ± 0.25	1.9 ± 0.01	0.19 ± 0.03	1.16 ± 0.08	0.23 ± 0.01

Table 2

Haze Measurements

	Beer	Beer + water	Beer + 25 LACU/liter	Beer + 50 LACU/liter
Air/bottle		3 ml / 6 ml	3 ml / 6 ml	3 ml / 6 ml
Initial haze, EBC	0.71	0.76 / 0.77	0.77 / 0.76	0.75 / 0.77
Final haze, EBC	0.77	0.85 / 0.86	0.80 / 0.90	0.80 / 0.82

Table 1 clearly demonstrates that Laccase is able to reduce the oxygen content significantly within a short period of time.

Immediately after bottling and prior to pasteurisation an oxygen content greater than 1.0 mg/liter is believed to form unwanted flavour in beer. The oxygen content can be reduced to below the acceptable level of 0.2 mg/liter in 1 hour by the Laccase treatment, thereby reducing detrimental chemical changes of the beer during storage.

The haze measurements in Table 2 show that the Laccase treatment has no significant influence on the formation of haze particles, which might reduce the shelflife of the beer, when measured using the quick shelflife test as described herein.

CLAIMS

1. A beer-making process, comprising
a) fermenting wort into beer, and
b) adding a laccase to the fermented beer so as to
5 improve the storage stability of the beer.

2. A beer-making process according to claim 1 in
which the storage stability of the beer is improved by reducing
the oxygen content.

3. A beer-making process according to claims 1-2, in
10 which the laccase is a microbial laccase.

4. A beer-making process according to claim 3, in
which the microbial laccase is derivable from a fungus, in
particular from a fungus belonging to the subdivision Basidio-
mycotina or the subdivision Ascomycotina.

15 5. A beer-making process according to claim 4, in
which the microbial laccase is derivable from a strain of
Aspergillus, Neurospora, Podospora, Botrytis, Collybia, Fomes,
Lentinus, Pleurotus, Trametes, Rhizoctonia, Coprinus, Psatyrel-
la, Myceliophthora, Schytalidium, Polyporus, Phlebia, Coriolus,
20 Hygrophoropsis, Agaricus, Vascellum, Crucibulum, Myrothecium,
or Sporormiella.

6. A beer-making process according to claim 5, in
which the microbial laccase is derivable from T. villosa,
T.versicolor or M. thermophila.

25 7. A beer-making process according to any of claims
1-6, wherein the amount of laccase is in the range from 0.1-
1000 LACU per liter of fermented beer, preferably in the range
from 1-50 LACU per liter of fermented beer.

8. A beer-making process according to any of claims

1-7, wherein polyvinylpolypyrrolidone is added to the fermented beer.